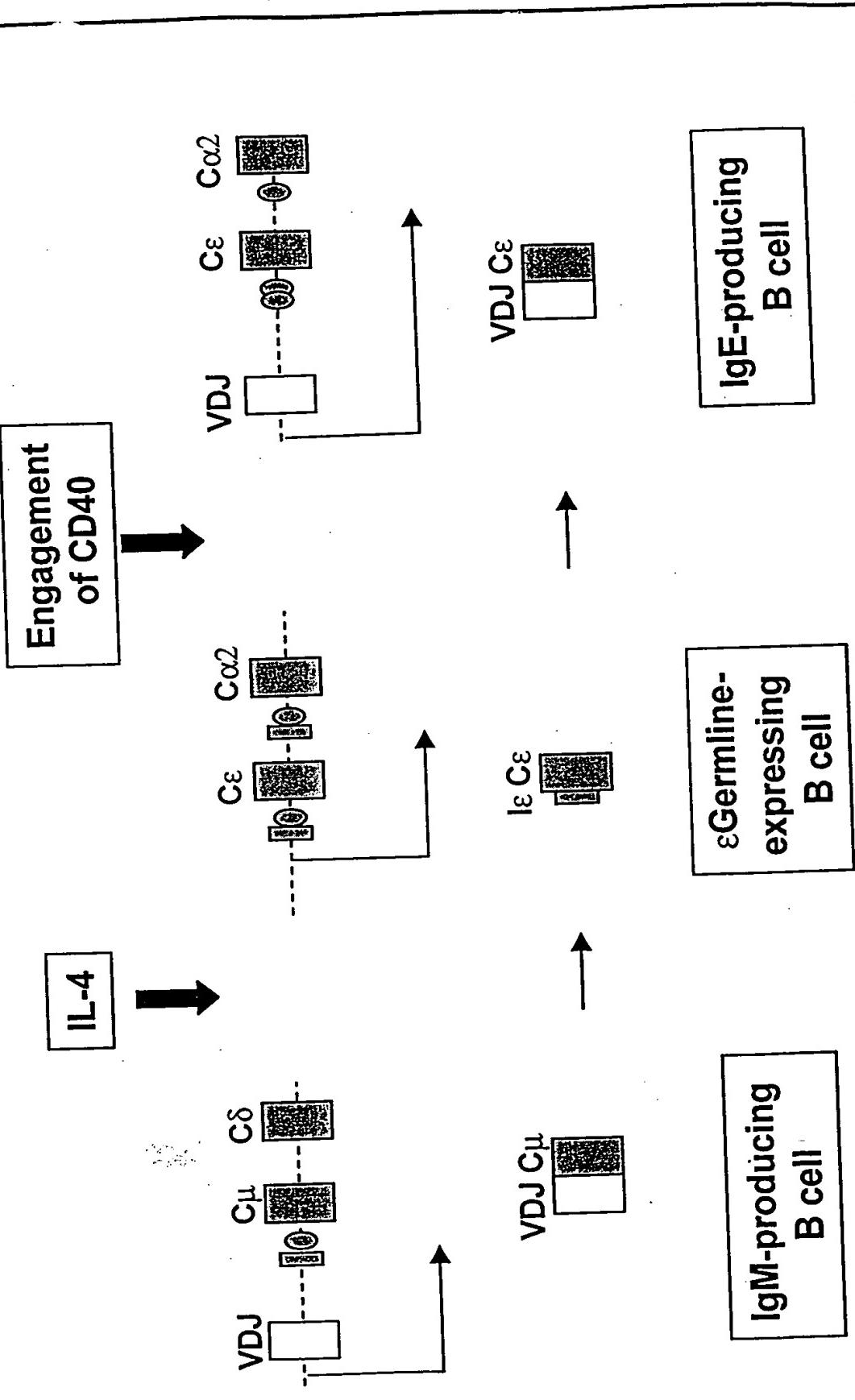


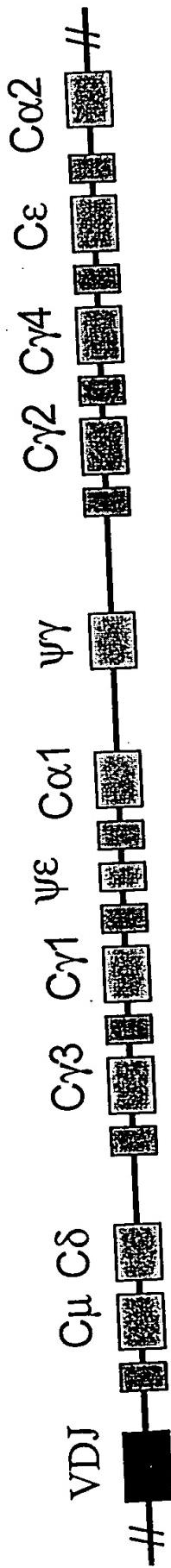
ε Germline Transcription and IgE Switching

FIGURE 1

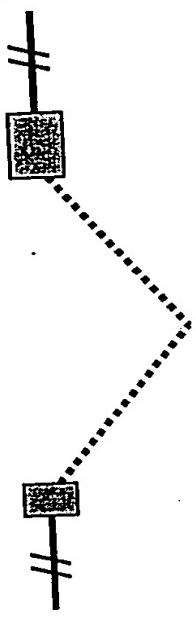


Chromosome 14 Human Heavy Chain Gene Map

FIGURE 2



Primary Germline Transcript



RNA Processing



■ = 1 exon

■ = Constant exons



Spliced Germline Transcript

FIGURE 3
1 OF 3

**Sequences of RPA Probes for Human Immunoglobulin
Germline Transcripts**

Germline Ig Alpha-2 Probe

CTCTGCTAAGGACAGACGGCCATCAAGGCAGGACCTGGGCCGGCCAGGGC
TCCCTCCCCACAGCAGCCCTCTGGCAGG
CAGCCAGACGCCGTGAGGGTGGACCTGCCATGAGGGCCTGCACGCCGGAG
GCCGCCACTCAGCACTGCCCTCCA
GCAGCCTGACCAGCATCCCCGACCAGCCCCAAGGTCTCCGCTGAGCCTCG
ACAGCACCCCCCAAGATGGGAACGTGGT
CGTCGCATGCCTGGTCCAGGGCTTCTCCCCCAGGAGCCACTCAGTGTGACCT
GGAGCGAAAGCGGACAGAACGTGACCG
CCAGAAAATCTCCCACCTAGCCAGGATGCCCTCCGGGACCTGTACACCACGAG
CAGCCAGCTGACCCTGCCGGCACACAG
TGCCCAGACGGCAAGTCCGTGACATGCCACGTGAAGCACTACACGAATCCA
GCCAGGATGTGACTGTGCCCTGCCAGT
TCCCCCACCTCCCCATGCTGCCACCCCCGACTGTCGCTGCACCGACCGGCC

Germline Ig Epsilon Probe

GGCTCCACTGCCCGGCACAGAAATAACAACCACGGTTACTGATCATCTGGGA
GCTGTCCAGGAACCCGACAGGGAGCCGG
ACGGGCCACACCACAGGCACCAAATGGACGACCCGGCGCTTCAGCCTC
CACACAGAGCCCATCCGTCTCCCCCTG
ACCCGCTGCTGAAAAACATTCCCTCCAATGCCACCTCCGTG

Germline Ig Gamma 1 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGGCTGGGAGCACGA
GGAACATGACTGGATGCCGGCAGAGCCGGC
CGTGGGGTGTGATGCCAGGATGGGCACGACCGACCTGAGCTCAGGAGGCAGCA
GAGCGAGGGAGGAGGAGAGGGCCCCAGGTG
AACGGAGGGCTTGTCCAGGCCGGCAGCATCACCGGAGCCCAGGGCAGGGT
CAGCAGTGCTGGCCGTGGGGCCCTCCT
CAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGGCCCATCGGTCTCCCCC
TGGCACCCCTCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTGTTGGAACTCAGGCGCCCTGA
CCAGCGCGTGCACACCTTCCGGCTGTCCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTACCGTGCCT
AGCAGCTTGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAAAGTTGAGCC
CAAATCTTGTGACAAAACACACATGCCACCG

FIGURE 3

2 OF 3

Germline Ig Gamma 2 Pr be

CCAAGCCAACAGGGCAGGACACACCAGAGGCTGACTGAGGCCTCCATGACG
ACCAGGGCTGGGAGCAGCAGGAACATGACG
GGATGCGGCAGAGCCGGCGTGGGTGATGCCAGCATGGGCAGGACCCACC
TGAGCTGAGGAGGCAGTAGAACGAGGGAG
GAGGAGAGGCCCAAGGTGAACGGAGGGCTTGTCCAGGCCAGCAGCATCAC
TGGAGCCCAGGGCAGGGTCAGCAGTGCTG
GCCGTGGGCCCTCTCTCAGCCAGGACCAAGGACAGCAGCCTCCACCAAGGG
CCCATCGGTCTTCCCCCTGGCGCCCTGC
TCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
ACTTCCCCGAACCGGTGACGGTGTGCGT
GAACTCAGGCCTCTGACCAGCGCGTGCACACCTCCAGCTGTCCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCG
TGGTACCGGTGCCCTCCAGCAACTTCGGCACCCAGACCTACACCTGCAACGT
AGATCACAAGCCCAGCAACACCAAGGTG
GACAAGACAGTTGAGCGCAAATGTTGTGTCGAGTGCCCACCGTGCCAGCAC
CACCTGTGGCAGGACCGTCA

Germline Ig Gamma 3 Probe

ACACACCAGAGGCTGACTGAGGCCTCCAGGACGACCGGGCTGGGAGCGTGA
GGAACATGACGGGATGGGGCAGAGCCAGC
CATGGGGTGTGCCAGGATGGGATGCCAGGACCTGAGCTCAGGAGGCAGCA
GAGAGAGGGAGGAGGAGAGGGCCCAAGGTG
AACCGAGGGCTTGTCCAGGCCGGCAGCATTACCGGAGCCCAGGGCAGGGT
CAGCAGAGCTGGCGTAGGGCCCTCTCT
CAGCCAGGACCAAGGACAGCAGCTCCACCAAGGGCCATCGGTCTTCCCCC
TGGGCCCTGCTCCAGGAGCACCTCTGG
GGGCACAGCGGCCCTGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTGCGTGGAACTCAGGCGCCCTGA
CCAGCGCGTGCACACCTCCGGCTGTCTACAGTCCTCAGGACTCTACTCC
CTCAGCAGCGTGGTACCGTGCCCTCC
AGCAGCTTGGCACCCAGACCTACACCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAGAGTTGAGCT
CAAAACCCACTTGGTACACAACTCACACATGCCACGGTGCCAGAGCCC
AAATCTTGTGACACACCTCCCCCGTGCC
CACGGTGCCC

FIGURE 3

3 OF 3

Germline Ig Gamma 4 Probe

GGCCAGCACCACATGGAAGCCAAGCGGAGCCAGCACGGGGAGGTGGCA
GCCTTCAGGCACTGATGCCACCCAGTGC
GAGACGACGGGACCCTGGCAGGGCTTCCAAGCCAACAGGGCAGGACAC
ACCAGAGGCTGACTGAGGCCTCCAGGACG
ACCGGGCTGGGAGCAGAGGAACATGACGGATGCGGCAGAACCGGCCGTG
GGGTGATGCCAGGATGGGCACGACCGACC
TGAGCTCAGGAGGCAGCAGAGCGAGGGAGGAGAGGCCAGGTGAACG
GAGGGGCTTGTCCAGGCCGGCAGCATCAC
CAGAGCCCAGGGCAGGGTCAGCAGAGCTGCCGTAGGGCCCTCCTCAGCC
AGGACCAAGGACAGCAGCTCCACCAAG
GGCCCATCCGTCTCCCCCTGGGCCCTGCTCCAGGAGCACCTCCGAGAGCA
CAGCCGCCCTGGCTGCCCTGGTCAAGGA
CTACTCCCCGAACCGGTGACGGTGTGGAACTCAGGCGCCCTGACCAGC
GGCGTGCACACCTCCGGCTGTCCCTAC
AGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTACCGTGCCCTCCAGCAG
CTTGGGCACGAAGACCTACACCTGCAAC
GTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCCAAA
TATGGTCCCCCGTC

FIGURE 4
1 OF 3

Sequences of RPA Probes for Human Immunoglobulin Germline Transcripts

Germline Ig Alpha-1 Probe

GGCTGGGGGCCAGGGCTCCCTCCCCACAGCAGCCTCTGGCAGGCAG
CCAGACGCCCCGTGAGGGTGGACCTGCCA
TGAGGGCCTGCACGCCGGAGGCCACTCAGCACTCGGGGCCCTCCAGCA
GCTGACCAGCATCCCCGACCAAGCCCCA
AGGTCTTCCCGCTGAGCCTCTGCAGCACCCAGCCAGATGGGAAACGTGGTCAT
CGCCTGCTGGTCCAGGGCTTCTTCCCC
CAGGAGOCACACTCAGTGTGACCTGGAGCGAAAGCGGACAGGGCGTGACCGCC
AGAAAATCCCCACCCAGCCAGGATGCCTC
CGGGGACCTGTACACCAAGGAGCAGCCAGCTGACCCCTGCGGCCACACAGTGC
CTAGCCGGCAAGTCGGTACATGCCAC

Germline Ig Alpha-2 Probe

CTCTGCTAAGGACAGACGGCCATCAAGGCAGGACCTGCGCCGGGCCAGGGC
TCCCCCCCCACAGCAGCCCTCTGGCAGG
CAGCCAGACGCCGTGAGGGTGGACCTGCCATGAGGG CTGCACGCCGGAG
GCCGCCCACTCAGCACTGCCGCCCTCCA
GCAGCCTGACCAGCATCCCCGACCAGCCCCAAGGTCT CCCGCTGAGCCTCG
ACAGCACCCCCCAAGATGGAACGTGGT
CGTCGATGCCGGTOAGGGCTCTCCCCCAGGAGC AACTCAGTGTGACCT
GGAGCGAAAGCGGACAGAACGTGACCG
CCAGAAAATCCCCACCTAGCCAGGATGCCCTCGGGGACCTGTACACCAACGAG
CAGCCAGCTGACCTGCCGCCACACAG
TGCCCCAGACGGCAAGTCCGTGACATGCCAC

Germline Ig Epsilon Probe

GGCTCCACTGCCGGCACAGAAATAACAACCACGGTTA CTGATCATCTGGGA
GCTGTCCAGGAACCGACAGGGAGCCGG
ACGGGCCACACCCATCCACAGGCACCAAATGGACGACCGCGCITCAGCCTC
CACACAGAGCCCATCCGTCTTCCCCITG
ACCCGCTGCTGCAAAAACATTCCCTCCAATGCCACCTCGTG

Germline Ig Gamma 1 Probe

ACACACCAGAGGCTGACTGAGGCCCTCAGGACGACCGKGCTGGGAGCACGA
GGAACATGACTGGATCGGGCAGAGCCGGC
CGTGGGGTGTGCCAGGATGGGCACGACCGACCTGAGKTCAGGAGGCAGCA
GAGCGAGGGAGGAGGAGAGAGGGCCCCAGGTG
AACGGAGGGGCTTGTCCAGGCAGGCATCACCGGAICCCAGGGCAGGGT
CAGCAGTGCTGGCGTGGGGCCCTCCTCT
CAGCCAGGACCAAGGACAGCAGCCTCACCAAGGGCICATCGGTCTTCCCCC
TGGCACCCCTCCCAAGAGCACCTCTGG
GGGCACAGCGGCCCTGGCTGGCTGGCAAGGACTACITCCCCGAACCGG

Germline Ig Gamma 2 Probe

CCAAGCCAACAGGGCAGGACACACCCAGAGGCTGACTGAGGCCTCCATGACG
ACCAAGGCTGGGAGCACGAGGAACATGACG
GGATGCGGGCAGAGCCGGCOGTGGGGTGTGCCAGCATGGCAGGACCCACC
TGAGCTGAGGAGGCAGTAGAACGAGGGAG
GAGGAGAGGCCCCAGGTGAACGGAGGGCTGTCCAGGCCAGCAGCATCAC
TGGAGCCCAGGGCAGGGTCAGCAGTGCTG
GCCGTGGGGCCTCTCTCAGCCAGGACCAAGGACAGCAGCTCCACCAAGGG
CCCATCGGTCTTCCCCCTGGCGCCCTGC
TCCAGGAGCACCTCCGAGAGCACAGCGGCCCTGGCTCCTGGTCAAGGACT
ACTTCCCCGAACCGG

Germline Ig Gamma 3 Probe

ACACACCAGAGGGCTGACTGAGGCTCCAGGACGACCGTGCTGGGAGCGTGA
GGAACATGACGGGATGGGCAGGCCAGC
CATGGGGTGTGACAGGATGGCATGACCGACCTGAGTCAGGAGGCAGCA
GAGAGAGGGAGGGAGGAGGAGAGGGCCCCAGGTG
AACCGAGGGGCTTGTCCAGGCCGGCAGCATCACCGGAACCCAGGGCAGGGT
CAGCAGAGCTGGCCGTAGGGCCCTCCCT
CAGCCAGGACCAAGGACAGCAGCTCCACCAAGGGCCATCGGTCTTCCCCC
TGGCGCCCTGCTCCAGGAGCACCTCTGG
GGGCACAGCGGCCCTGGGCTGCCCTGGTCAAGGACTAC.TCOCGGAAACGGTG
ACGGTGTGTTGGAACTCAG

Germline Ig Gamma 4 Probe

GGCCAGCACCATGGAAGCCCCAAGCGGAGCCAGCAC
GCCTTCAGGCACTGATGCCACCCAGTGC
GAGACGACGGGGACCCTGGGCAGGGGCTTCCAAGCCA
ACAGGGCAGGACAC
ACCAAGAGGCTGACTGAGGCCTCCAGGACG
ACCGGGCTGGGAGCACGAGGAACATGACGGGATGCGG
CAGAACCGGCCGTG
GGGTGATGCCAGGATGGGCACGACCGACC
TGAGCTCAGGAGGCAGCAGACGAGGGAGGAGAG
GCCCCAGGTGAACG
GAGGGGCTGTCCAGGCCGGCAGCATCAC
CAGAGCCCAGGGCAGGGTCAGCAGAGCTGGCGTAGG
GCCCTCCTCTCAGCC
AGGACCAAGGACAGCAGCTTCCACCAAG
GGCCCATCCGTCTCCCCCTGGCGCCCTGCTCCAGGAG
CACCTCCGAGAGCA
CAGCCGCOCTGGGCTGCTGGTCAAGGA
CTACTTCCCOGAACCGG

FIGURE 5

RPA PROBES

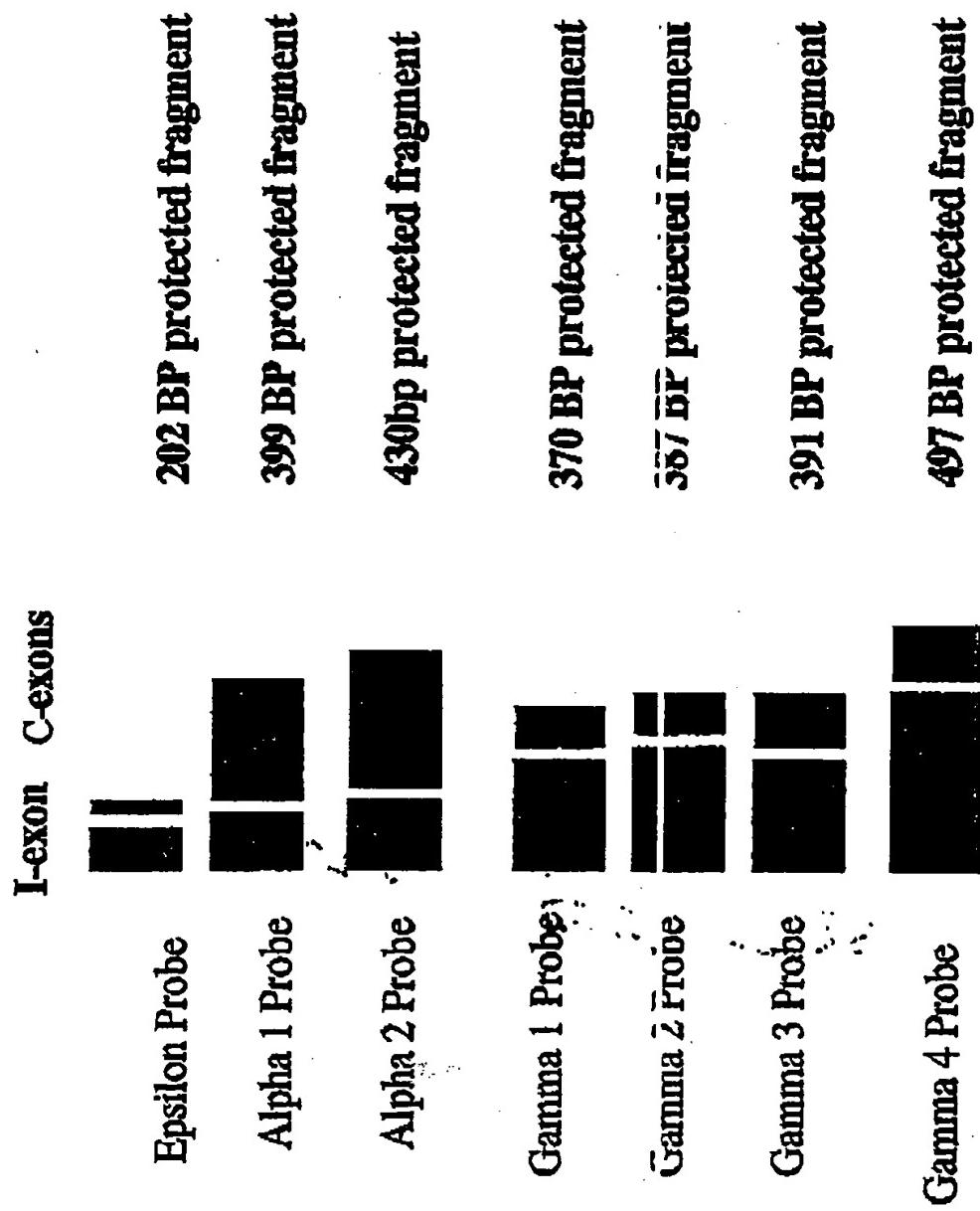
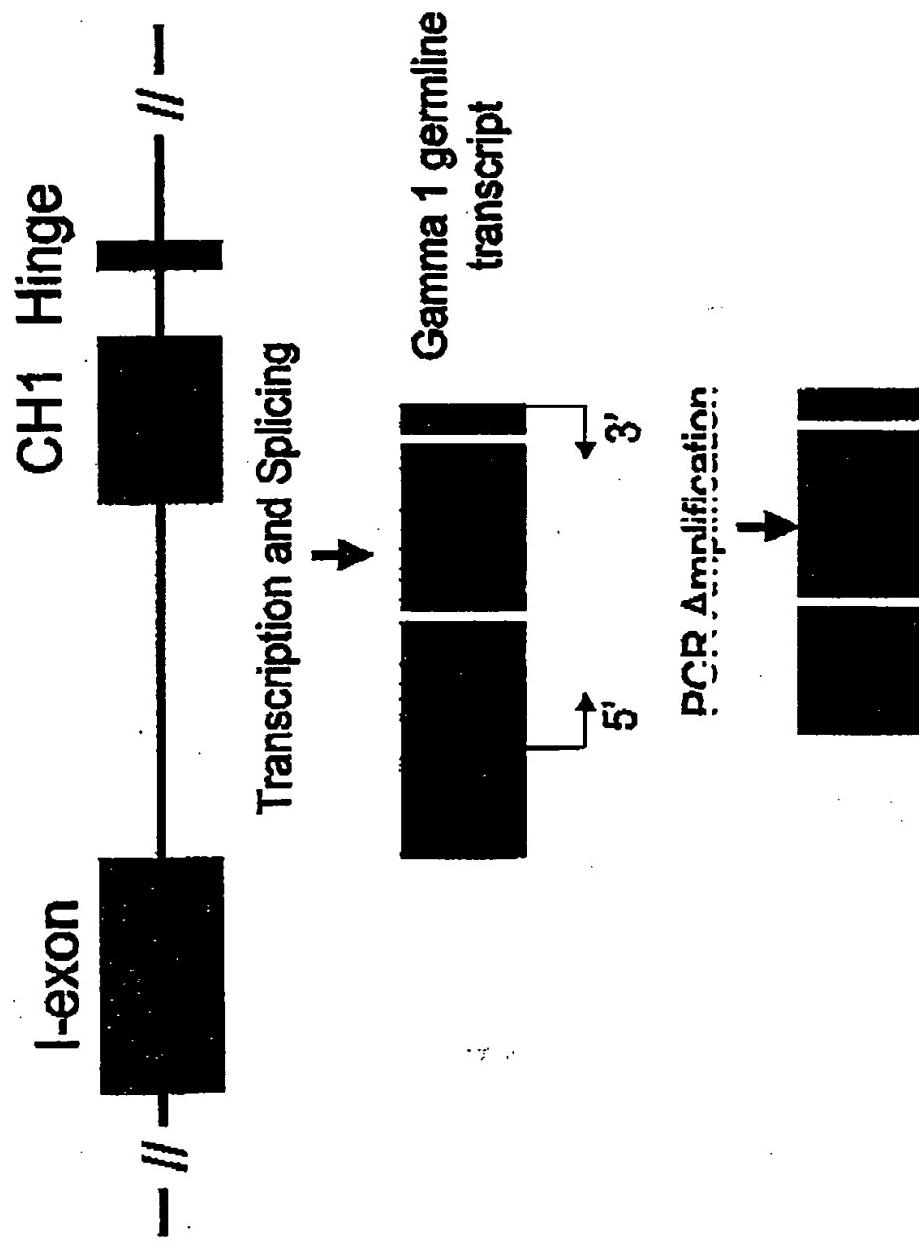


FIGURE 6

Gamma 1 Probe



The Gamma 1 5' and 3' Primers amplified a completed probe of 370 BP

RNAse Probe Protection Assay

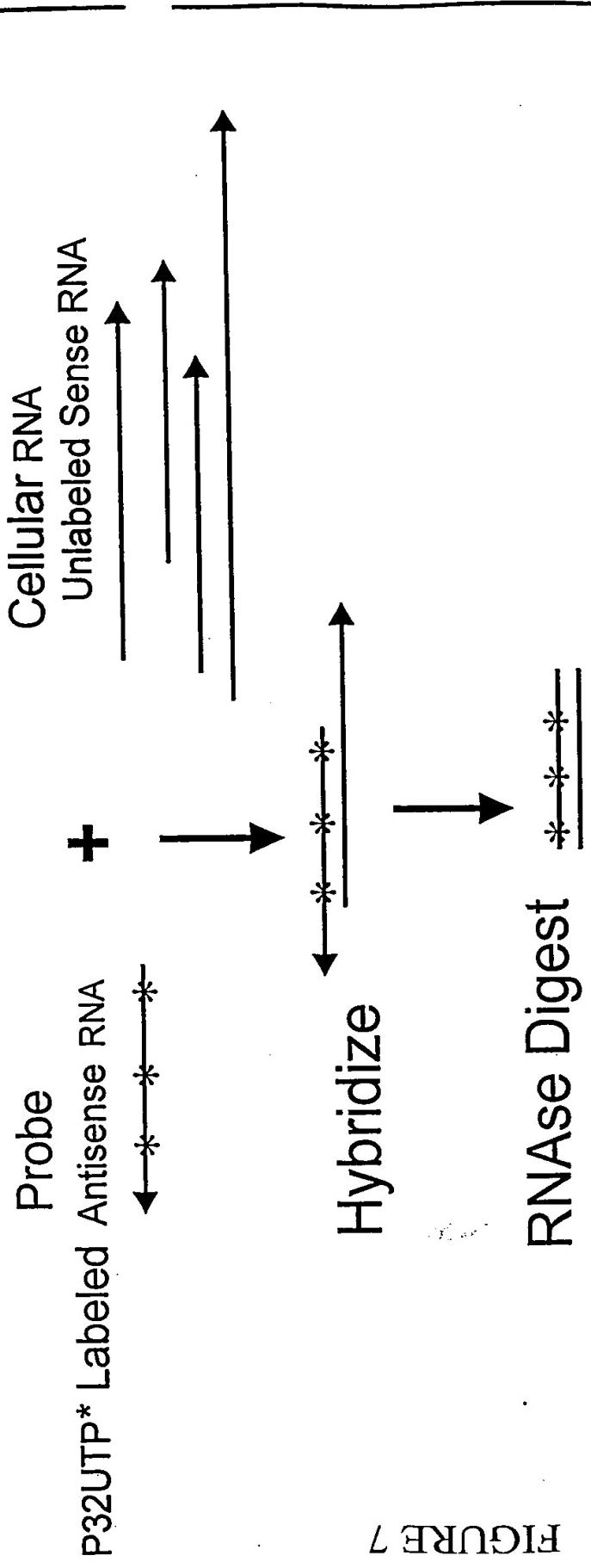
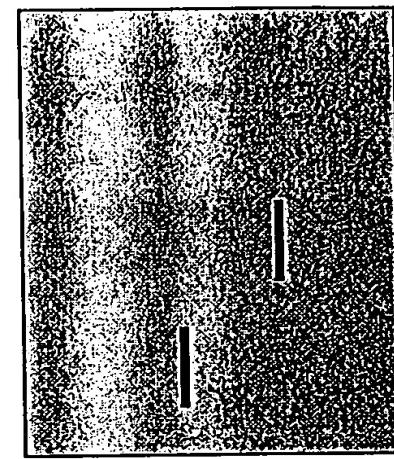


FIGURE 7



Run undigested probe vs digested protected fragment on acrylamide-Urea gel

Undigested Probe

Protected Fragment

Visualize using beta imaging equipment



Promega

Technical Bulletin

FIGURE 8

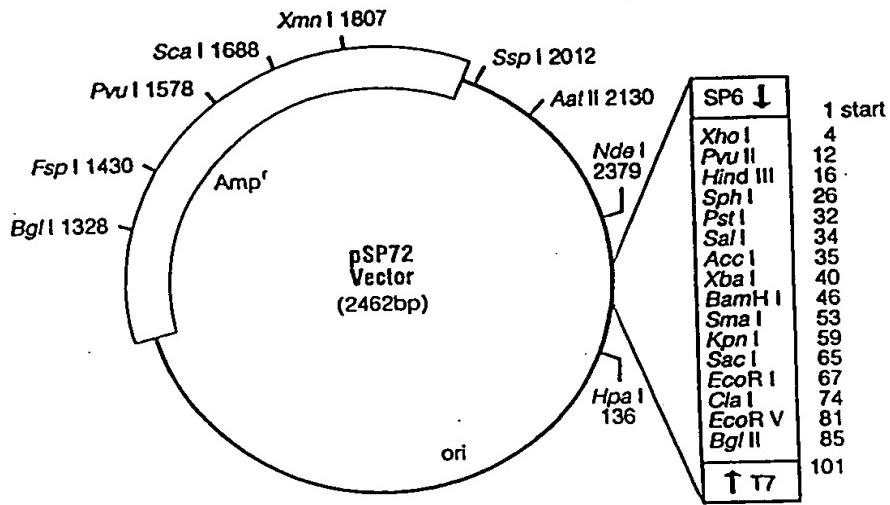


Figure 2. pSP72 Vector circle map and sequence reference points.

1. Sequence reference points:
 - a. SP6 RNA polymerase transcription initiation site 1
 - b. T7 RNA polymerase transcription initiation site 101
 - c. SP6 RNA polymerase promoter 2446-6
 - d. T7 RNA polymerase promoter 96-118
 - e. multiple cloning sites 4-90
 - f. β-lactamase (Amp^r) coding region 1135-1995
2. Specialized application:
 - a. transcription *In vitro* from dual opposed promoters (For protocol information, please request Promega's Riboprobe® *In vitro* Transcription Systems Technical Manual, #TM016.)
3. The pSP72 and pSP73 Vectors are identical except for the orientation of the multiple cloning region.
4. Blue/white screening for recombinants is not possible with the pSP72 Vector.

FIGURE 9

Accession Numbers for Germline Transcripts

Alpha - 1

L04541 = I Region Exon
BC005951 = Constant Region Exon

Alpha - 2

L04541 = I Region Exon
AL389978 = Constant Region Exon

Epsilon

X56797 = I Region Exon
J00222 = Constant Region Exon

Gamma - 1

AL122127 = I Region Exon
Z17370 = Constant Region Exon

Gamma - 2

U39934 = I Region Exon
J00230 = Constant Region Exon

Gamma - 3

AL122127 = I Region Exon
X16110 = Constant Region Exon

Gamma - 4

X56796 = I Region Exon
K01316 = Constant Region Exon